SOME STUDIES ON THE FACTORS OF PATHOGENIC DETERMINANTS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM BOVINE MASTITIS

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By

Saad El-Said Amer Kotb

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بعض الدراسات عن عوامل الضراوة لميكروب المكور العنقودى الذهبى المعزول من إلتهاب الضرع فى الماشية

رسالة علمية

مقدمة إلى الدراسات العليا بكلية الطب البيطرى – جامعة الأسكندرية

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فى

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مقدمة من

ط.ب./ سعد السيد عامر قطب

٢٠١٠

Advisors' Committee

Prof. Dr. Mohamed Ali Akiela

Professor of Microbiology

Head of Microbiology Department

Faculty of Veterinary Medicine

Alexandria University

Prof. Dr. Riad Hassan Khalil

Professor of Fish and Crustacean Diseases

Head of Poultry and Fish Diseases

Department

Faculty of Veterinary Medicine

Alexandria University

لجنة الإشراف

الأستاذ الدكتور/ محمد على عقيلة

أستـــاذ الميكروبيولوجيا

رئيس قسم الميكروبيولوجيا

كلية الطب البيطرى

جامعة الأسكندرية

الأستاذ الدكتور/ رياض حسن خليل

أستـــاذ أمراض الأسماك و القشريات

رئيس قسم أمراض الدواجن و الأسماك

كلية الطب البيطرى

جامعة الأسكندرية

بسم الله الرحمن الرحيم

وما أوتيتم من العلم إلا قليلاً

صدق الله العظيم

سورة الإسراء الآية رقم ٨٥

Dedicated To
My Parents
My Sisters & My Brother

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LIST OF ABBREVIATIONS

٢-DE	٢-D electrophoresis
APHA	American Public Health Association
bp	Base pair
CLFa	Clumping factor
CMT	California mastitis test
CNS	Coagulase negative staphylococci
Coa	Coagulase gene
CPS	Coagulase positive staphylococci
CRF	Coagulase reacting factor
DDW	Double-distilled water
dNTPS	Deoxynucleotide triphosphate solution
EDTA	Ethylene diamine tetra-acetic acid
ET	Exfoliative toxin
Fnb	Fibronectin binding A
FSF	Fibrin stabilizing factor
hla	Alpha-haemolysin
hlb	Beta-haemolysin
IMI	Intramammary infection
Luk	Leukocidin gene
MLST	Multilocus sequence typing
mPCR	Multiplex polymerase chain reaction
nuc	Thermonuclease gene
O-F	Oxidation-Fermentation
PAGE	polyacrylamide gel electrophoresis.
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
RFLP	Restriction fragment length polymorphism
RPLA	Reverse passive latex agglutination assay
rRNA	Ribosomal ribonucleic acid
S.	Staphylococcus
SCC	Somatic cell count
SDS	Sodium dodecyle sulphate
SE	Staphylococcal enterotoxin
SEA	Staphylococcal enterotoxin A
Sp.	Species
Spa	Staphylococcal protein A
Str.	Streptococcus
TCT	Tube coagulase test
TE	Tris-EDTA
TM	Melting temperature
TPE	Tris-phosphate EDTA
TSST-I	Toxic shock syndrome toxin-١
Tth	Thermus thermophilus

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INTRODUCTION

Worldwide bovine mastitis is the most common infectious disease affecting milk producing cows causing economic losses higher than any other disease of dairy cattle ***(Gillespie and Oliver, ٢٠٠٥)***. Also, it possesses food safety and anti-microbial resistance threats ***(Kim et al., ٢٠٠١),*** as it is the primary contamination source of milk and milk products especially in case of defective pasteurization ***(Joffe and Baranovics, ٢٠٠٦)***.

The major causes of bovine mastitis are *Staphylococcus (S.) aureus, Str. agalactiae, Str. dysgalactiae, Str. ubris* ***(Phuektes et al., ٢٠٠١)***. *S. aureus* is recognized worldwide as a major pathogen causing subclinical intramammary infections in dairy cows ***(Salasia et al., ٢٠٠٤)***.

Identification of bacterial pathogens in milk from cows with mastitis is the definitive diagnosis of mastitis infections. It also provides information important for prevention and control of the disease. In most clinical laboratories identification methods are based on microbiological culture of milk and biochemical identification of bacterial isolates recovered. However, these microbiological cultures are limited by the dynamic nature of infections. Subclinically infected cows are intermittent shedders of organisms through low and high shedding patterns during lactation leading to negative cultures ***(Phuektes et al., ٢٠٠١)***.

In bovine mastitis, coagulase positive staphylococci (CPS) (*S. aureus* and some strains of *S. hyicus*) are common pathogenic than coagulase negative staphylococci (CNS) isolates ***(Ali-Vehmas and Sandholm, ١٩٩٥)***. The TCT has the potential to detect not only *S. aureus* but also other CPS in milk.

The ability of *S. aureus* to form clumps in the presence of plasma has been known since the turn of the century ***(Much, ١٩٠٨)***. Staphylococcal components which interact with fibrinogen and can be purified from *S. aureus* culture supernatant fluids have been described. These include a staphylocoagulase and a cell wall protein (clumping factor, CLFa) which binds with fibrinogen ***(Boden and Flock, ١٩٩٢)***. Staphylocoagulase, produced by *S. aureus*, form an active molecular complex with prothrombin which converts fibrinogen to a fibrin clot ***(Hendrix et al., ١٩٨٣)***. Studies of gene sequences have demonstrated that coagulase and CLFa factor are genetically distinct ***(McDevitt et al., ١٩٩٢)***. Detection of staphylocoagulase and CLFa are used for identification of *S. aureus* in diagnostic laboratories ***(Kloos and Schleifer, ١٩٨٦).*** Molecular epidemiological analysis of bovine *S. aureus* population suggested that a small numbers of clonal types were responsible for most infections, and that strains had a broad geographic distribution ***(Kalorey et al., ٢٠٠٧).*** A better knowledge on the distribution of *S. aureus* in dairy animals might help formulate strategies to reduce the spread of infection ***(Salasia et al., ٢٠٠٤)***.

The pathogenic staphylococci produce a "battery" of toxins and enzymes ***(Quinn et al., ١٩٩٤)*** which make a contribution to the ability of this organism to cause disease on the mammalian host ***(Salasia et al., ٢٠٠٤).*** These exotoxins include haemolysins, various enzymes and a family related pyrogenic toxins, namely staphylococcal enterotoxin (SE), toxic shock syndrome toxins (TSST) and exfoliative toxins (ET) ***(Dinges et al., ٢٠٠٠)***. Recently a novel gene cluster encoding staphylococcal exotoxins-like protein has been described ***(Williams et al., ٢٠٠٠)***.

The analysis of relationship between presence/absence of the different genes and the udder inflammatory response measured by milk somatic cell count (SCC) showed that at least one cluster, which made by classification of isolates in cluster by virulence genes, induced a higher inflammatory response. Moreover, the analysis of the association between virulence genes and the presence of subclinical mastitis, support that it could be related to strain characteristics, and the expression of specific combination of virulence factor specially the spa (staphylococcal protein A), COA (coagulase gene) and SEJ (one of the SEs) ***(Zecconi et al., ٢٠٠٦).*** No remarkable difference was recognized in the identification ratio of the isolates which belonged to the two major lineages between mastitis of subclinical origin and clinical origin ***(Hata et al., ٢٠٠٦)***.

This work aimed to study some factors of the pathogenic determinants of *S. aureus* isolated from clinical bovine mastitis. This will be accomplished through:

١. Isolation and classification of staphylococci recovered from samples collected from clinically mastitis cows examined.

٢. Determining the incidence of *S. aureus* in milk samples collected from cases of bovine clinical mastitis.

٣. Studying certain pathogenic determinants of *S. aureus* recovered from clinical mastitis milk. Moreover, identification of *S. aureus* enterotoxin (SEA) by using PCR.